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New insights into probiotic mechanisms

A harvest from functional and metagenomic studies

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There has been continued and expanding recognition of probiotic approaches for treating gastrointestinal and systemic disease, as well as increased acceptance of probiotic therapies by both the public and the medical community. A parallel development has been the increasing recognition of the diverse roles that the normal gut microbiota plays in the normal biology of the host. This advance has in turn has been fed by implementation of novel investigative technologies and conceptual paradigms focused on understanding the fundamental role of the microbiota and indeed all commensal bacteria, on known and previously unsuspected aspects of host physiology in health and disease. This review discusses current advances in the study of the host-microbiota interaction, especially as it relates to potential mechanisms of probiotics. It is hoped these new approaches will allow more rational selection and validation of probiotic usage in a variety of clinical conditions.

Introduction

The field of probiotics—defined as "live microorganisms that, when administered in sufficient amounts, confers a health benefit on the host" (Food and Agriculture Organization of the United Nations, 2002)—continues to evolve as many laboratories are employing cutting edge methodology to investigate the complex interactions of the myriad potential probiotic agents with an equally dizzying array of potential health benefits.[1,2] Indications for probiotic use have historically focused on GI disease, as digestive disorders are substantial causes of morbidity and mortality in humans. These conditions range from infectious gastroenteritis and functional disturbances (e.g., irritable bowel syndrome), to immuno-inflammatory disorders (e.g., ulcerative colitis and Crohn disease) and long-term chronic conditions (e.g., colorectal cancer).[3] Additionally, recent work has also identified putative roles for the gut microbiota in the etiology of systemic conditions like the metabolic syndrome and autistic spectrum disorders, which are thus potentially treatable by probiotic approaches.[4,5] Overall, the rationale for continued study of the therapeutic benefits of probiotics remains strong. This review explores the emerging studies demonstrating how the intestinal microbiota and its novel genetic endowment impacts human physiology, suggesting roles that probiotics might play directly or indirectly on modifying these functions.

It is increasingly being recognized that the human microbiome, including those organisms comprising the residents of the oral cavity, skin and especially the gastrointestinal tract, has a profound impact on normal physiology and health. For example, in vitro experiments and studies with germ free animals have convincingly demonstrated that a healthy intestinal microbiota mediates important roles in normal gut gene regulation and homeostasis, influencing epithelial growth and survival, innate and adaptive immune development and regulation, restitution after injury and competitive exclusion of pathogens. Overall, the theoretical rationale for probiotic use is based on exploitation of physiological mechanisms by which the prokaryotic microbiota interact among themselves, influence the intestinal epithelia and immune system and conversely, how the host manages the microbiota while defending itself from the (comparatively) rare pathogen. A thorough understanding of the gut microbiome and individual probiotic species is required to effectively plan intervention studies.[6] Therefore, a focus of recent investigation has been the cataloging of genetic and metabolic diversity of the microbiota—both as an ecological accounting of an important symbiotic system and also as a practical search for candidate exploitable microbial strains and biochemical functions. Additionally, investigators continue to elucidate mechanisms of immunological cross-talk among members of the microbiota and the role of microbiota on human physiology and development. Finally, genetic analysis has increased our knowledge of the biochemical attributes of individual probiotic species.

Functional Genomics of Candidate Probiotics

Comparative genomic analyses of the archetypal probiotics, lactobacilli and bifidobacteria, which occupy either food/dairy systems or colonized regions of the human body, have identified both conserved and unique gene sets important for biochemical/metabolic functions. Of the probiotic microbes studied extensively to date, the lactobacilli have been found most amenable to genetic manipulation and functional analysis of specific genes and operons. Functional genomics have identified systems responsible for acid and bile tolerance and prebiotic transport and metabolism.

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By performing microarray studies, a set of approximately 125 genes (approximately 5–6% of the *L. reuteri* genome) is suggested to contribute to mucosal and systemic immune responses. These genes have been placed in the context of cell signaling and metabolic modeling using bio-informatics approaches including metabolic modeling.[7] In addition, a substantial number of cell surface proteins and structures have been identified on probiotic microbes that interact directly with host epithelial cells and immunomodulatory cells. Among these are lipoteichoic acids (LTA), surface layer proteins, mucus binding proteins and a pilus expressed by *Lactobacillus rhamnosus* that also promotes binding to mucus.[8,9] Recent studies have shown that genetic alteration or elimination of the cell surface display of such structures, notably LTA and Slayer proteins, can dramatically alter dendritic cell binding and cytokine signaling and promote inflammatory or anti-inflammatory responses. In the case of LTA from lactobacilli, elimination of the molecule or alteration of its charge, dramatically changes cytokine signaling in immunomodulatory cells from an inflammatory to an anti-inflammatory display and results in a probiotic microbe able to mitigate inflammation, colon cancer and colitis in mouse models.[10,11,53]

The ability of probiotic microbes to survive gastric transit and interact intimately with the intestinal epithelium has provided new opportunities to deliver biotherapeutics and notably vaccines, to the intestinal mucosa.[12] Considerable success has been realized recently with *Lactobacillus acidophilus* and *Lactobacillus gasseri* used to express the protective antigen from anthrax. Administered orally, these engineered bacteria elicit protective immunity to mice.[11] The efficacy of the vaccine is due in large part to the C-terminal fusion of a targeting peptide of 12 amino acids, which promotes binding of the vaccine to dendritic cells in the intestinal mucosa.

The *Lactobacillus* genus is comprised of over 150 species and has been divided into major clades, based on phylogenetic analysis. One important group is the *Lactobacillus salivarius* clade, which has been subject to significant genome sequencing and genetic characterization over the past 5 years.[13] The identification of novel, strain-specific properties of *L. salivarius* were revealed by in-depth genome sequencing and comparative genome hybridization of 33 different strains. One important genetic feature in this species is a circular mega-plasmid that appears ubiquitous and is likely a reservoir for horizontally transferred genes, the most notable example being the broad spectrum bacteriocin, salivaricin. In this presentation, predicted adhesins encoded by both chromosome and plasmid determinants were described, including fibrinogen and mucus binding proteins, respectively. Interestingly, 50% of the *L. salivarius* strains encode a fibrinogen binding protein (SrfA), but few express a phenotype. An insertional knockout mutant of the novel gene for this fibrinogen receptor in strain CCUG47825 showed loss of fibrinogen binding.

Comparative genomic analysis between *L. salivarius* and other intestinal lactobacilli also identified an additional cell-surface determinant on the surface of "motile" lactobacilli, specifically *Lactobacillus ruminis* strains. Annotation of the *L. ruminis* ATCC 27782 genome identified all the motility and motility-associated proteins required to produce a fully functional flagellar apparatus; 45 predicted proteins involved in regulation, synthesis, export and chemotaxis behavior.[14] *L. ruminis* exerts significant immunomodulatory properties, including stimulation of tumor necrosis factor (TNF) and nuclear-factor (NFκB) production in monocytes.

"Niche factors" that promote the survival, retention and activity of microbes in the GI tract are at the same time identified as virulence factors for pathogens and colonization factors for commensals/probiotics. In *Bifidobacterium breve*, recent genome sequencing and analysis identified a Type IV pili, which was found previously in some Gram-negative pathogens.[15] The Tad pilus-encoding locus was conserved among other *B. breve* strains supporting the notion of a ubiquitous pili-mediated host colonization and persistence mechanism for bifidobacteria, representing a niche factor that is clearly shared with intestinal pathogens. A second example is bile tolerance and its importance for intestinal survival to both pathogens and probiotics. Introduction of a bile tolerance operon (bilE) from *Listeria monocytogenes* into *B. breve* was shown to significantly improve this probiotic microbes' tolerance to bile and resident colonization ability. Pathogens and probiotics/commensals share critical niche factors that underlie their survival in similar environments. In this regard, new studies on probiotic mechanisms will likely benefit from decades of previous research on pathogens.

Bifidobacterium species are among the major members of the beneficial commensal microbiota and are among the first microbes to colonize the gastrointestinal tract of breast fed infants. Specific oligosaccharides within human breast milk are abundantly produced in the lactation cycle and are preferentially used by *Bifidobacterium longum* subsp *infantis* to promote its colonization of the developing infant. Comparative genomic analysis of this species identified a novel 40 kb region that appeared dedicated to human milk oligosaccharide (HMO) utilization. Functional characterization of the cluster of HMO genes revealed a complex metabolic pathway dedicated to transport and metabolism of these specific oligosaccharides,[16] which selectively promotes the growth of *B. infantis*[17]—including the increased production of genes which increase anti-inflammation and tight junction proteins and decrease inflammation.[52] This gene cluster is inducible and uniquely expressed during growth by this microbe on HMO's. Such conditions also promote binding of *B. infantis* to intestinal epithelial cells and stimulation of endocrine cells. This research builds off an understanding of the basic molecular chemistry of HMO's leading to an enhanced understanding of the interactions and ecological consequences of this symbiosis that promotes stable development of mutualistic bifidobacteria in the infant.

Functional Genomics of the Microbiota

The microbiome itself is the totality of a mixed community of microorganisms (the microbiota), including its genetic components and the resulting functionality. The ultimate objective of global microbiome research effort is to explore associations of the bacterial species, communities, genes, genomes in the human gut

with various clinical states (e.g., obesity and inflammatory bowel diseases), though a cause or effect relationship remains far from clear. At the species and community level, bacterial 16 S rRNA sequencing based taxonomic analysis of the human microbiota has revealed a diverse and dynamic community of approximately 10^{14} prokaryotic organisms with a biomass of greater than 1 kg, comprising over 4000 separate species, with a large majority of the population are representatives of two divisions, the *Bacteroidetes* and *Firmicutes*.[18] The population composition is remarkably stable at different anatomic locations along the gut, but absolute numbers vary greatly, ranging from 10^{11} cells per gram content in the ascending colon, 10^{7-8} in the distal ileum and 10^{2-3} in proximal ileum and jejunum. Additionally, metagenomics—bulk genomic sequencing of entire communities/environments—allows inventories of the entire microbiota component genes, which can be tabulated and correlated with host phenotypes. For example, pyrosequencing of total fecal DNA from 124 individuals of European origin revealed 3.3 million non-redundant genes, 150-fold more than encoded by our own genome. Interestingly 3 groups "biotypes" of individuals were identified as based on microbial enterotype. In this study, metagenome analysis of a large number of individuals from four countries revealed three robust clusters of microorganisms (enterotypes) as well as balanced host microbial symbiotic states which are not related to age, gender, or body mass index but may functionally respond differently to diet and to drug intake.[19] These enterotypes have recently been studied in relationship to long-term diet and have shown a correlation.[20] Additionally, recent evidence suggests that crosstalk between lymphocytes, microbiota and the intestinal epithelium can affect either immunity vs. metabolism in the gut.[21] Genetic control of these functions may explain responder and non-responders in probiotic and prebiotic trials (where the microbiome composition is influenced by dietary intervention and host factors.[22] Thus, publication of first drafts of the human microbiome project led to novel insights in nutrient extraction, gene function, xenobiotic processing, metabolic regulation and the development of mucosal interactions **Figures 1, 2 and Table 1.**

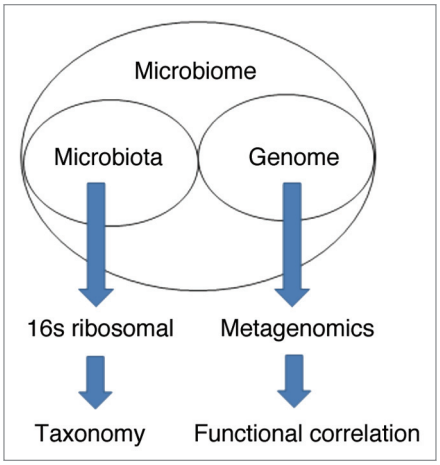

Figure 1. Approach for analysis of the microbiota. Methods to interrogate 16s ribosomal sequences allow taxonomic study of bacterial populations. Bulk sequencing of whole bacterial genomes allow analysis of the functional capabilities of the microbiome.

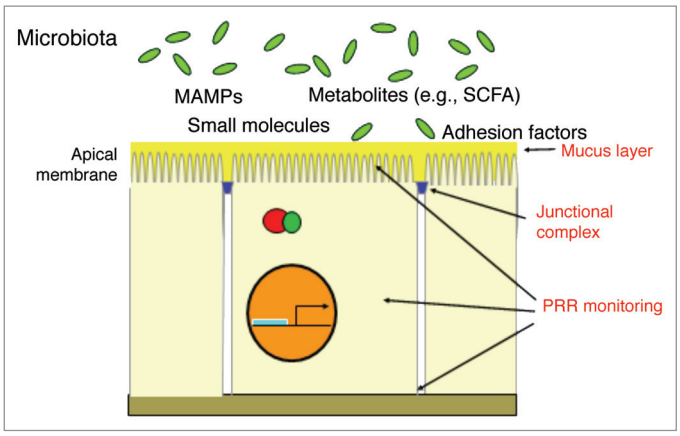

Figure 2. Cartoon of the intestinal interface with the microbiota and by extension, with probiotics. For details, see text.

The Intestinal Interface with the Microbiota and Probiotics

The dividing line between commensal and pathogen can be very fine, even dualistic. Certain taxa have been implicated as "pathobionts," meaning usual members of the microbiota that can be opportunistic pathogens under certain circumstances. For example, *Clostridium difficile* is a common resident of the microbiota that is responsible for pseudomembranous colitis. This acute inflammatory colitis generally develops following broad spectrum antibiotic administration and the presumed disruption of normal microbiota ecological structure that allows *C. difficile* to overgrow. Clinical experience has shown strong, if anecdotal, evidence that microbiota reconstitution via fecal transplant may be an important therapeutic modality in this disorder.[23]

Both pathogens and probiotics are interlopers into a commensalistic microbiota that could originate from the GI microbiota, or be introduced or selected via dietary and pharmacological interventions, such as prebiotics or antibiotics.[24] Pathogens and probiotics must overcome identical barriers to survive gastric

Table 1. Proposed effects of probiotics

Competitive exclusion of pathogens
Altered metabolism/energy utilization
Stimulation of intestinal motility
Adaptive immune development and regulation
Innate immune regulation
Dampening of inflammatory responses
Stimulation of redox signaling
Epithelial development and survival
Cytoprotective effects of PRR signaling
Stimulation of barrier function, epithelial restitution

transit, resist the antimicrobial effects of bile, compete with existing microbiota and ultimately interact with the intestinal mucosa, with the initial point of contact at the epithelia. Epithelial cells have long been understood as a primary physical barrier against pathogens, forming a selective cellular barrier and are now recognized as a principal interface with the microbiota, initiating innate and adaptive immune responses to both.[25] Essentially all the physical and near physical contact between host and microbe occurs at the epithelial (or epithelial derived, e.g., mucus layer) surfaces. Epithelial cells, by embryological definition, are interfaces between the host and the environment (in a topological sense, the gut lumen is external to the body) and are equipped with a number of structural and biochemical modifications to mediate this function. Interestingly, certain probiotic strains of *Bifidobacterium infantis* and *Lactobacillus plantarum* have the capacity to enhance gut barrier function through altering gene expression and distribution of occludin, ZO-1, ZO-2 and different claudin isoforms.[26] Another fundamental property of the epithelia is their ability to monitor bacterial presence, which is generally recognized to involve host perception of prokaryotic macromolecular motifs (such as LPS, peptidoglycan and flagellin, among others) that are bound and recognized by pattern recognition receptors, such as TLRs and Nod proteins.[27] These macromolecules, designated MAMPs (microbial associated molecular patterns) are generally invariant structural components of the bacteria. MAMP bound PRRs result in activation of signaling pathways eventuating in host gene regulatory events that likely have broadly cytoprotective effects and with quantitative or qualitatively increased stimulation, result in cellular inflammation and apoptosis. High throughput transcriptional profiling has been used to determine the immunoregulatory factors are responsible for inflammatory or cytoprotective mediation.[28] It is well established that low levels of tonic stimulation of the mucosal by MAMPs via TLR signaling is necessary for normal growth and homeostasis of the gut mucosa and probiotics may possess species-specific components that signal host cells in addition of generally recognized MAMPs. For example, a soluble protein, p40, derived from *Lactobacillus rhamnosus* GG has been purified and cloned and shown to have anti-inflammatory activity in model murine colitis.[29] Another example has been reported whereby a structural *Bacteroides fragilis* cell wall component, an exopolysaccharide, PSA, reproduced the anti-inflammatory effects of the parent bacteria in vitro and in vivo.[30]

There is increasing evidence that bacterially produced small molecules have a role in the host-microbe crosstalk. The generation of microbial metabolites as a result of amino acid conversion results in the production of immunoregulatory, low molecular weight signals that enable bacteria to suppress mucosal immunity and enhance nutrition. A variety of small compounds or metabolites are being characterized from different commensal microbes. Many probiotic organisms are involved in fermentation and produce metabolic products such as indole (promotes barrier function[31]), acetate, which can protect against *E. coli* 0157 infection,[32] and, as will be discussed, butyrate an energy source for epithelium. Microbial derived small molecules may have signaling functions. In multi-cellular organisms intercellular communication may occur through hormonal signaling. Unicellular organisms, such as bacteria also engage in chemical signaling, termed quorum sensing, to coordinate population-wide behavior, mimicking multi-cellular organisms. In addition, inter-kingdom signaling also occurs between uni- and multi-cellular organisms, mediating amicable and detrimental interactions. For example, the bacterial pathogen enterohemorrhagic *E. coli* (EHEC), responsible for outbreaks of bloody diarrhea worldwide, exploits cell-to-cell signaling between the gastrointestinal microbiota and the host as a means to gauge and recognize the host environment. This inter-kingdom signaling is predicated upon hormonal communication and utilizes the host epinephrine and/or norepinephrine (NE) stress hormones and a bacterial aromatic hormone-like signal named autoinducer-3 (AI-3).[33] Small molecule networks, with origins both from prokaryotic and host sources may be a component of the optimal ecology of a healthy microbiota.

Another class of soluble small molecule includes reactive oxygen species (ROS). Bacterial induction of ROS is a cardinal feature of the phagocyte response to bacteria and indeed is a feature of bacterial response in virtually all animals and plants. Recent work has shown commensal bacteria rapidly stimulate the production of reactive oxygen species in the intestinal epithelia.[34] In mammalian cells, ROS serve as critical second messengers in multiple signal transduction pathways via the rapid and transient oxidative inactivation of a range of regulatory enzymes, including those involve in NFκB activation, MAPK signaling and cytoskeletal dynamics. There are marked differences in the relative ability of different commensal microorganisms to induce ROS and these events are mediated by components of the bacterial cell wall, suggesting a dedicated cellular receptor (communication). This rapid response may represent another highly conserved mechanism of host-microbiota cross talk.

The microbiota is clearly involved in the anatomic and functional development of mucosal adaptive immunity mediated by intraepithelial lymphocytes and immunomodulatory cells resident in the mucosal lamina propria. In germ free animals, Peyer's patches are grossly hypoplastic, IgA responses are reduced and diminished total CD4 T cell populations and an inappropriate balance of T_H cell subsets are seen.[35] Many gut microbes, including probiotics, can manipulate the host's adaptive immune system through secretion of "immunomodulins" that affect cell-signaling pathways in mammalian cells. Some of these effects are direct on immune cells such as dendritic cells (DC) which can themselves protrude through intact epithelial tight junctions and sample luminal contents and even engulf bacteria. Many probiotic bacteria promote the generation and upregulation of regulatory DC and T cells and this is associated with increased production of the regulatory cytokines TGFβ and especially IL10. The latter is synthesized by a variety of cell types including the epithelium and has been shown to be neuroactive and inhibitory in models of visceral pain.[36]

Interestingly, unlike the non-species-specific mechanisms of bacterial recognition that affect innate immune and epithelial processes, adaptive immune stimulation seems to be predominantly regulated by distinct species. For example, the T-cell abnormalities in germ free mice can be rectified within weeks

upon colonization with a representative member of the normal bacteria (*Bacteroides fragilis)* via dendritic cell recognition of a specific polysaccharide (Polysaccharide A) component of *B. fragilis*.[30] Interestingly, the intestinal lamina propria in healthy animals was shown to be a major location of a unique population of interleukin-17 producing $CD4^+$ T-cells (T_H17 cells) distinct from Th1 or Th2 cell lineages. These T-cells seem to be distinctly stimulated by a single bacterial taxon, the segmented filamentous bacteria (SFB), a non-culturable spore forming Gram-positive clostridia-related species, further implicating this taxon as a primary driver of adaptive immune development.[37,58] In addition, a recent publication provides evidence that *Bifidobacterium breve* induces IL-10 producing Tr1 cells in the colon by activating dendritic cells which then produce IL-10 and IL-17 which in turn activate naïve T cells to develop into IL-10 producing Tr1 cells.[54] In other words, probiotic manipulation of the adaptive immunity may require fine-tuned selection of biologically relevant strains.

Finally, the microbiota and probiotics can influence functional GI processes. The enteric nervous system (ENS) innervates the entire intestine sending neurites to the villous tips so that neuroimmune bi-directional communication is constantly occurring. Nerve growth factor (NGF) synthesis is promoted in the intestinal epithelium by lactobacilli and has an autocoid relationship with IL10, the former promoting the synthesis of the latter,[38,55] further showing the importance of neuroimmune interactions in maintaining gut homeostasis. Several days of feeding probiotics also alters the function of the ENS so that ingested lactobacilli affect a specific ion channel, the intermediate calcium-activated potassium channel.[39] In a model of peristalsis, the same bacteria had a similar effect, slowing the frequency of neural dependent muscle contractions within minutes of luminal contact in a dose dependent fashion.[40] ENS to brain communication is also being affected by ingestion of probiotic bacteria as 28 d of feeding produced anxiolytic changes in behavior, reflected by alterations of the expression of GABA receptors in the brain and attenuated stress responses, all of which were abrogated by prior subdiaphragmatic section of the vagus nerve.[41] Thus, further extensive study of the microbiome-gut-brain axis can be expected to bring insight into some of the mechanisms involved.

Influence of the Microbiota on Metabolism and Nutrition

Recent studies using metagenomics and metabolomics approaches have highlighted the complex inter-relationship between the microbiota and mammalian metabolism and have shown that the gut microbiota play an important role not only in the way we derive energy from our diet but also in the way we store this energy.[42-46] This has been recently studied in the context of the unique microbiota in obesity with animal models and human subjects,[47] using a combination of gnotobiotic and metagenomic techniques.[48] When the microbiota of an obese human are given to a lean gnotobiotic mouse, the animal gains weight at a faster rate than its litter mate on the same caloric intake.[49] This is also true when microbiota from monozygotic lean/obese twins are used. The same observation has been made when the microbiota of twins from Malawi with or without severe malnutrition were given to gnotobiotic mice and maintained on a Malawian diet.[50] Under these conditions, the mice lost weight until they were changed to a high calorie diet. These observations suggest that diet influences the collective microbiota, which in turn maintain the human phenotype when the original dietary intake persists. Interesting results were also obtained when exogenous, commercial bacteria added to the diets were used in an attempt to alter the microbiota of gnotobiotic mice colonized with human microbiota.[49] Four different diets, comprised of protein, fat, starch or sucrose, were fed along with the putative probiotic bacteria. Specific changes in the composition and function of these bacteria were noted with each diet, suggesting that colonizing bacteria adapt to the energy source provided in their diet for their own survival. These observations suggest that modification of the gut microbiota based on specific phenotypes (e.g., obesity vs. malnutrition) can provide possible new approaches to altering disease using specific symbiotic organisms. However, these observations must be made in the context of clinically accepted multi-center trials.

There is no greater example of a dietary influence on the composition of colonizing gut bacteria than the differences between infants fed exclusively by breast milk vs. formula feeding. During the age period of nascent colonization, the composition of breast milk, particularly its complex carbohydrates (oligosaccharides), strongly influences the levels of *Bifidobacterium* and *Lactobacillus*, important symbiotic microorganisms involved in the development of intestinal immune homeostasis.[17,52] Principally, a modified diet induces endogenous microbes to multiply based on their ability to metabolize otherwise indigestible dietary carbohydrates. The microbiota also produce essential nutrients according to the infants' needs, such as increased folate in early infancy and increased vitamin B12 in later infancy.

Mechanistically, the microbiota can influence nutrition and weight gain by contributing to energy extraction, a process enabled by the novel enzymatic functions provided by non-eukaryotic genomes in the microbiota. Specially, distinct strains of bacteria can ferment substrates otherwise unavailable as a caloric source to the human hosts. The two main types of fermentation that are performed in the gut are saccharolytic and proteolytic. The main products of saccharolytic fermentation are the short chain fatty acids, acetate, propionate and butyrate. All contribute to the host's daily energy requirements. Acetate is metabolized in systemic areas like muscle and used to generate ATP, while propionate may be transported to the liver. Butyrate is a source of energy for colonocytes and has touted anti-neoplastic properties. The end products of proteolytic fermentation on the other hand, include toxic metabolites such as amines and ammonia. The concept of fermentation of novel substrates forms the basis of "prebiotic" approaches. A prebiotic is a non-digestible food ingredient that beneficially affects the host by targeting indigenous beneficial components of the microbiota. Main prebiotic targets at the moment are bifidobacteria and lactobacilli (although this may change as knowledge of the microbiota diversity and functionality expands). Recent studies have also suggested that not only do prebiotics affect proliferation of probiotic-like bacteria, but

also preferentially stimulate microbial gene products which affect regulatory immune function.[52] Any dietary component that reaches the colon intact is a potential prebiotic. However much of the interest in the development of prebiotics is aimed at non-digestible oligosaccharides such as fructooligosaccharides (FOS) and galactooligosaccharides (GOS and trans-GOS). Although human studies on prebiotics are increasing, the field lacks feeding trials that document the long-term impact of prebiotic-induced gut microbiota modulation on human physiology.

Conclusion

Overall recent advances in our understanding of the role to the microbiota and microgenome in host biology will profoundly alter our approach to the identification, optimization and validation of probiotics agents and clinical indications. For example, metabolomic studies in mice have demonstrated that treatment with probiotic strains alters metabolism both within the intestinal tract as well as systemically and further, that these alterations in metabolism are linked with significant changes within the colonic microbiome. Taken in the context of live animals or humans, the overall balance of individual strains or species of microbes within the intestine contributes enormously to gut homeostasis. Conversely, imbalance or dysbiosis within the microbiome leads to local and systemic disturbances associated with ill health and disease. Additionally, although a unique individualized microbiome is a result of multiple factors [genetic and environmental (diet, antibiotic use and mode of delivery at birth)], the diet given to a person over a prolonged period of time strongly impacts their microbiota and appears to reduce the incidence of a changing disease paradigm from infections to immune-mediated disease reported worldwide.[45] Finally, as differences in microbial profiles from various disorders are delineated, the overall goal will be to improve diagnostic markers and develop robust mechanisms of microbiota modulation (such that the "healthy" state is achieved).[28] Approaches that encompass ecological theory, statistics, molecular-based assessments of diversity, host response and clinical markers will improve our understanding of health through the gut microbiota.[21,28] Overall, the application of modern "omic" technologies is expected to advance the art and science of probiotics and stimulate realization of their promised benefits.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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